d.) Remarks

Any inquiry concerning this communication or earlier communications from the applicant should be directed to Chuan Li whose telephone number is (858) 361-7231. The applicant can normally be reached from 9:00 a.m. to 5:00 p.m. pacific standard time.

The applicant may also be reached at Expression Technologies Inc. at (858) 558-1861 or by fax at (858) 558-1883 or by email at chuanli@exptec.com.

Applicant Name: Chuan Li

Signature: 

Date: December 17, 2009

Sequence ID NO: 1

BIO•SYNTHESIS

Lot No: B716-1

Oligo Data Sheet

Date Created:	**3/11/98**
Your Reference ID:	**OLIGO 1** 1C2C0131
Primer Lot Number:	**B716-1**
Author:	**MD**
Synthesis Scale:	**50 nmole**
Primer Sequence (5' to 3'):	**CGC CCG CCG CCC GGG CGC CCC GCC TTC CGC TTC CTC GCT CAC TG**

Primer Data

Primer Length:			44		
Type:			DNA		
Composition:	A	C	G	T	Others
	1	25	11	7	0
	2.3%	56.8%	25.0%	15.9%	0.0%

Molecular Weight (Ammonium Salt):	13231.8
Exact Weight per OD (Ammonium Salt):	37.87
Nanomoles per OD (Ammonium Salt):	2.86
Micromolar Extinction Coefficient:	349.38
Total ODs in This Tube:	5
Total Amount in ug:	189.36
Total Amount in nmoles:	14.31
Purification:	Desalted
Melting Temperature in Celsius:	160.0

5' END	OH
3' END	OH

Note: OD WILL VARY

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Sequence ID NO: 24

BIO•SYNTHESIS

Lot No: B716-3

Oligo Data Sheet

Date Created:	**3/11/98**
Your Reference ID:	**OLIGO 3** IC2C0133
Primer Lot Number:	**B716-3**
Author:	**MD**
Synthesis Scale:	**50 nmole**
Primer Sequence (5' to 3'):	**CGC CCG CCG CCC GGG CGC CCC GCC AAC GCG GAA GTC AGC GCC CT**

Primer Data

Primer Length:			44		
Type:			DNA		
Composition:	A	C	G	T	Others
	5	23	14	2	0
	11.4%	52.3%	31.8%	4.5%	0.0%

Molecular Weight (Ammonium Salt):	13372.8
Exact Weight per OD (Ammonium Salt):	34.85
Nanomoles per OD (Ammonium Salt):	2.61
Micromolar Extinction Coefficient:	383.67
Total ODs in This Tube:	5
Total Amount in ug:	174.27
Total Amount in nmoles:	13.03
Purification:	Desalted
Melting Temperature in Celsius:	162.0

5' END	OH
3' END	OH

Note: OD WILL VARY

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Sequence ID NO: 3

BIO•SYNTHESIS

Lot No: B716-4

Oligo Data Sheet

Date Created:	**3/11/98**
Your Reference ID:	**OLIGO 4** 10,2C 0331
Primer Lot Number:	**B716-4**
Author:	**MD**
Synthesis Scale:	**50 nmole**
Primer Sequence (5' to 3'):	**CGC CCG CCG CCC GGG CGC CCC GCC AAC GCA GAC CGT TCC GTG GC**

Primer Data

Primer Length:			44		
Type:			DNA		
Composition:	A	C	G	T	Others
	4	23	14	3	0
	9.1%	52.3%	31.8%	6.8%	0.0%

Molecular Weight (Ammonium Salt):	13363.8
Exact Weight per OD (Ammonium Salt):	35.38
Nanomoles per OD (Ammonium Salt):	2.65
Micromolar Extinction Coefficient:	377.73
Total ODs in This Tube:	5
Total Amount in ug:	176.9
Total Amount in nmoles:	13.24
Purification:	Desalted
Melting Temperature in Celsius:	162.0

5' END	OH
3' END	OH

Note: OD WILL VARY

Sequence ID NO: 4

BIO•SYNTHESIS

Lot No: B716-5

Oligo Data Sheet

Date Created:	**3/11/98**
Your Reference ID:	**OLIGO 5** 3C015
Primer Lot Number:	**B716-5**
Author:	**MD**
Synthesis Scale:	**50 nmole**
Primer Sequence (5' to 3'):	**CCG CCG CGC CGC TTC CAC TGA GCG TCA GAC CC**

Primer Data

Primer Length:	32
Type:	DNA

Composition:

A	C	G	T	Others
4	16	8	4	0
12.5%	50.0%	25.0%	12.5%	0.0%

Molecular Weight (Ammonium Salt):	9668.4
Exact Weight per OD (Ammonium Salt):	34.97
Nanomoles per OD (Ammonium Salt):	3.62
Micromolar Extinction Coefficient:	276.48
Total ODs in This Tube:	5
Total Amount in ug:	174.85
Total Amount in nmoles:	18.08
Purification:	Desalted
Melting Temperature in Celsius:	112.0

5' END	OH
3' END	OH

Note: OD WILL VARY

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Sequence ID NO: 5

BIO•SYNTHESIS

Lot No: B716-7

Oligo Data Sheet

Date Created:	**3/11/98**
Your Reference ID:	**OLIGO 7** IGAN
Primer Lot Number:	**B716-7**
Author:	**MD**
Synthesis Scale:	**50 nmole**
Primer Sequence (5' to 3'):	**GGG CGG CGG GCG TTC GGG GAA ATG TGC GCG GA**

Primer Data

Primer Length:			32		
Type:			DNA		
Composition:	A	C	G	T	Others
	4	6	18	4	0
	12.5%	18.8%	56.3%	12.5%	0.0%

Molecular Weight (Ammonium Salt):	10068.4
Exact Weight per OD (Ammonium Salt):	31.85
Nanomoles per OD (Ammonium Salt):	3.16
Micromolar Extinction Coefficient:	316.08
Total ODs in This Tube:	5
Total Amount in ug:	159.27
Total Amount in nmoles:	15.82
Purification:	Desalted
Melting Temperature in Celsius:	112.0

5' END	OH
3' END	OH

Note: OD WILL VARY

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Sequence ID NO: 6

BIO•SYNTHESIS

Lot No: B716-8

Oligo Data Sheet

Date Created:	**3/11/98**
Your Reference ID:	**OLIGO 8** IGKN
Primer Lot Number:	**B716-8**
Author:	**MD**
Synthesis Scale:	**50 nmole**
Primer Sequence (5' to 3'):	**GGG CGG CGG GCG TTG TCG GGA AGA TGC GTG AT**

Primer Data

Primer Length:			32		
Type:			DNA		
Composition:	A	C	G	T	Others
	4	5	17	6	0
	12.5%	15.6%	53.1%	18.8%	0.0%

Molecular Weight (Ammonium Salt):	10058.4
Exact Weight per OD (Ammonium Salt):	31.95
Nanomoles per OD (Ammonium Salt):	3.18
Micromolar Extinction Coefficient:	314.82
Total ODs in This Tube:	5
Total Amount in ug:	159.75
Total Amount in nmoles:	15.88
Purification:	Desalted
Melting Temperature in Celsius:	108.0

5' END	OH
3' END	OH

Note: OD WILL VARY

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Sequence ID NO: 7

BIO•SYNTHESIS

Lot No: B716-9

Oligo Data Sheet

Date Created:	**3/11/98**
Your Reference ID:	**OLIGO 9** IGTN'
Primer Lot Number:	**B716-9**
Author:	**MD**
Synthesis Scale:	**50 nmole**
Primer Sequence (5' to 3'):	**GGG CGG CGG GCG TTC TCA TGT TTG ACA GCT TA**

Primer Data

Primer Length:	32
Type:	DNA

Composition:

A	C	G	T	Others
4	7	12	9	0
12.5%	21.9%	37.5%	28.1%	0.0%

Molecular Weight (Ammonium Salt):	9903.4
Exact Weight per OD (Ammonium Salt):	33.11
Nanomoles per OD (Ammonium Salt):	3.34
Micromolar Extinction Coefficient:	299.07
Total ODs in This Tube:	5
Total Amount in ug:	165.57
Total Amount in nmoles:	16.72
Purification:	Desalted
Melting Temperature in Celsius:	102.0

5' END	OH
3' END	OH

Note: OD WILL VARY

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Sequence ID NO: 8

BIO•SYNTHESIS

Lot No: B716-10

Oligo Data Sheet

Date Created:	**3/11/98**
Your Reference ID:	**OLIGO 10** IGCN
Primer Lot Number:	**B716-10**
Author:	**MD**
Synthesis Scale:	**50 nmole**
Primer Sequence (5' to 3'):	**GGG CGG CGG GCG AAG CCA CTG GAG CAC CTC AA**

Primer Data

Primer Length:			32		
Type:			DNA		
Composition:	A	C	G	T	Others
	7	10	13	2	0
	21.9%	31.3%	40.6%	6.3%	0.0%

Molecular Weight (Ammonium Salt):	9910.4
Exact Weight per OD (Ammonium Salt):	31.42
Nanomoles per OD (Ammonium Salt):	3.17
Micromolar Extinction Coefficient:	315.45
Total ODs in This Tube:	5
Total Amount in ug:	157.08
Total Amount in nmoles:	15.85
Purification:	Desalted
Melting Temperature in Celsius:	110.0

5' END	OH
3' END	OH

Note: OD WILL VARY

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Sequence ID NO: 9

BIO•SYNTHESIS

Lot No: B716-11

Oligo Data Sheet

Date Created:	**3/11/98**
Your Reference ID:	**OLIGO 11** 3GAC
Primer Lot Number:	**B716-11**
Author:	**MD**
Synthesis Scale:	**50 nmole**
Primer Sequence (5' to 3'):	**GCG GCG CGG CGG TAC GGG GTC TGA CGC TCA GT**

Primer Data

Primer Length:			32		
Type:			DNA		
Composition:	A	C	G	T	Others
	3	9	15	5	0
	9.4%	28.1%	46.9%	15.6%	0.0%

Molecular Weight (Ammonium Salt):	9939.4
Exact Weight per OD (Ammonium Salt):	33.32
Nanomoles per OD (Ammonium Salt):	3.35
Micromolar Extinction Coefficient:	298.26
Total ODs in This Tube:	5
Total Amount in ug:	166.62
Total Amount in nmoles:	16.76
Purification:	Desalted
Melting Temperature in Celsius:	112.0

5' END	OH
3' END	OH

Note: OD WILL VARY

Sequence ID NO: 10

BIO•SYNTHESIS

Lot No: B716-12

Oligo Data Sheet

Date Created:	**3/11/98**
Your Reference ID:	**OLIGO 12** 3GKC
Primer Lot Number:	**B716-12**
Author:	**MD**
Synthesis Scale:	**50 nmole**
Primer Sequence (5' to 3'):	**GCG GCG CGG CGG ATC GCC CCA TCA TCC AGC CA**

Primer Data

Primer Length:			32		
Type:			DNA		
Composition:	A	C	G	T	Others
	5	14	10	3	0
	15.6%	43.8%	31.3%	9.4%	0.0%

Molecular Weight (Ammonium Salt):	9757.4
Exact Weight per OD (Ammonium Salt):	33.61
Nanomoles per OD (Ammonium Salt):	3.44
Micromolar Extinction Coefficient:	290.34
Total ODs in This Tube:	5
Total Amount in ug:	168.03
Total Amount in nmoles:	17.22
Purification:	Desalted
Melting Temperature in Celsius:	112.0

5' END	OH
3' END	OH

Note: OD WILL VARY

Sequence ID NO: 11

BIO•SYNTHESIS

Lot No: B716-13

Oligo Data Sheet

Date Created:	**3/11/98**
Your Reference ID:	**OLIGO 13** 3GTC
Primer Lot Number:	**B716-13**
Author:	**MD**
Synthesis Scale:	**50 nmole**
Primer Sequence (5' to 3'):	**GCG GCG CGG CGG TTC ACG TTC GCT CGC GTA TC**

Primer Data

Primer Length:	32
Type:	DNA

Composition:

A	C	G	T	Others
2	11	12	7	0
6.3%	34.4%	37.5%	21.9%	0.0%

Molecular Weight (Ammonium Salt):	9825.4
Exact Weight per OD (Ammonium Salt):	34.87
Nanomoles per OD (Ammonium Salt):	3.55
Micromolar Extinction Coefficient:	281.79
Total ODs in This Tube:	5
Total Amount in ug:	174.34
Total Amount in nmoles:	17.74
Purification:	Desalted
Melting Temperature in Celsius:	110.0

5' END	OH
3' END	OH

Note: OD WILL VARY

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Sequence ID NO: 12

BIO•SYNTHESIS

Lot No: B716-14

Oligo Data Sheet

Date Created:	**3/11/98**
Your Reference ID:	**OLIGO 14** 3GCC
Primer Lot Number:	**B716-14**
Author:	**AH**
Synthesis Scale:	**50 nmol**
Primer Sequence (5' to 3'):	**GCG GCG CGG CGG AAG CAC ACG GTC ACA CTG CT**

Primer Data

Primer Length:			32		
Type:			DNA		
Composition:	A	C	G	T	Others
	6	11	12	3	0
	18.8%	34.4%	37.5%	9.4%	0.0%

Molecular Weight (Ammonium Salt):	9861.4
Exact Weight per OD (Ammonium Salt):	32.27
Nanomoles per OD (Ammonium Salt):	3.27
Millimolar Extinction Coefficient:	305.55
Total ODs in This Tube:	5
Total Amount in ug:	161.37
Total Amount in nmoles:	16.36
Purification:	Desalted
Melting Temperature in Celsius:	110.0

5' END	OH
3' END	OH

Note: OD WILL VARY

↓ 14K 10'

↓ 1ml 70% EtOH

↓ 2'

↓ air dry

pellets

The Amount of DNA of #2 and #4 products is about 18ug.
Amount of DNA of #7, #8, #9 and #4 products is about 28 ug.
90ul and 140ul H_2O to make their final concentration at about 0.2 ug/ul.
Store them at −20°C.

4 PCR OS 6-19-98

Now I am doing PCR analysis since the PCR Test worked fine.

DNA	Primers	Product	bps	Comments
pBR322, PstI	3+5	01	1535	
"	1+5	02	813	
pUC19, RI	1+5	03	813	
pACYC177, BHI	4+5	04	1011	
"	1+5	05	726	
pBR322, PstI	2+5	06	1057	
pACYC177, BHI	1+6	07	699	
"	4+6	08	979	
pACYC184, BHI	1+6	09	694	
pBR322, PvuII	7+11	S1	1130	
pUC19, RI	7+11	S2	1130	
pACYC177, BHI	7+11	S3	1130	
"	8+12	S4	1219	
pBR322, PvuII	9+13	S5	1552	
pACYC184, BHI	10+14	S6	1104	

DNA are from page 6. They are diluted to 1 ng/ul and 1 ul was for each reaction.
Primers are from page 5. The estimated concentration is about 8/ul. 2 ul of each primer was used.
Dilute the DNA into appropriate concentration.
Making master mix as on page 6 except the number of reactions should be 16 instead of 12. One extra is for negative

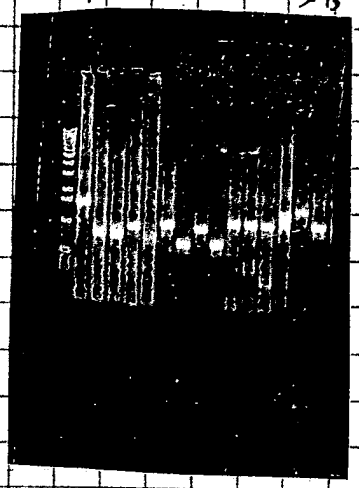
To Page No. 9

Read & Understood by me,	Date	Invented by	Date
	6/18/98		6-19-98
		Recorded by	

Page No. 8 control.

The PCR condition is same as on page 7.

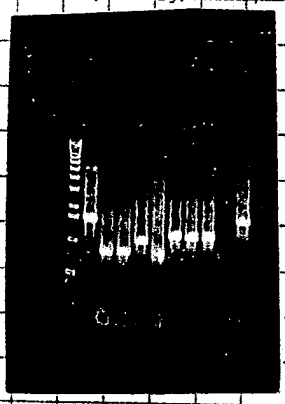
100 ul of each PCR product
↓ take out 3ul
6/24/98 ↓ run on 0.8% agarose TBE
← ↓ take a picture



All the reactions worked. However, some worked better than others. #1 to 5 and 10 to 14 seems have many non specific products. By increasing the template concentration, specific product may be increased and non specific products may be decreased.

6-20-98 → Repeat reactions #1 to 5 and 10 to 14 by increasing the template 10X (use 10ng)

100 ul of each PCR product
↓ take out 3ul
↓ ran on 0.8% agarose TBE
6/24/98 ← ↓ take a picture



All the reactions appear to be better except #13. I probably make mistake when adding template or primers for #13.
Keep the PCR products at -20°C

5. PCR SI 6-21-98

DNA	Amount	Primers	P	bps	Comments
pACYC177 BH1	1 ng	8+12	S4	1219	
"	10 ng	"	"	"	
pACYC177 PstI	1 ng	"	"	"	
"	10 ng	"	"	"	
pGEX-3X	1 ng	15+17	I	1198	

The primers are from page 5. 2 ul of each primer was used.
The PCR condition is same as on page 7.

To Page No. 10

Used & Understood by me,
STEVEN F. GESSERT
Date 6/24/98

Invented by Chuanli
Recorded by Chuanli
Date 6-20-98, 6-21-98

Page No. 92

② By comparing the yields of the miniprep's between page 11 and 92, it is clearer that the copy number of plasmids is determined by the selection marker it contains. In OS4 miniprep, the A based plasmids give very low yields except #15.

③ ~~The~~ SmaI has two sites on OS4 constructs. Therefore two bands generated after SmaI digestion. However the size of the DNA appear to be different even from the colonies ~~are~~ picked from same plate. This observation may be artifacts of electrophoresis, but I need pay attention on this observation in further analysis.

22. OS Mediprep Test 1 9.8.98

Use the residual ON cultures from 4 ml innoculation (These residual cultures are kept at 4°C). to seed 5 ml LB with appropriate antibiotic. prep number, page and ON culture number are indicated in the following table.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	84	84		87	87	85	87	85		91		91		92		
	2	28			18	22	21	25				16	2	8	10	15
	Amp				Tet				Chl				Kan			

The above table is messy. I will re-prepare the table below:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Amp						Tet				Chl				Kan	
	84	84		87	87	85	87	85	91		87	91		92		
	2	28	16	4	18	22	21	25	19	8	13	16	2	8	10	15

5 ml innoculated LB w/ appropriate antibiotic

↓ 37°C w/ shacking at 300 RPM for 3 hrs

1 to 4, 5, 7, 13 and 15 grows fast.
6, 8, 9 to 12, 14 and 16 grow slowly.

↓ 37°C for another 3h w/ shacking

9 to 12, 14 and 16 still do not grow well

↓ 37°C w/ shacking o/n. ~13hs.

9.9.98 innoculate all 5 ml into 50 ml LB w/ antibiotic

↓ 37°C for 1h w/ shacking

Test OD590

#1 : 0.435 #12 : 0.278

To Page No. 94

Used & Understood by me,	Date	Invented by	Date
	9-11-98	Chuan Li	9.8.98
		Recorded by Chuan Li	

↓ 37°C for another 1h w/ shaking.

Test OD575

#1 : 0.718 #12 : 0.283

#8 : 0.676 #16 : 0.257

The final concentration of chl 5×35/50 ≈ 10 ug/ml

↓ add 15 ul 35mg/ml chl to 1-8 and 13-16

↓ 37°C w/ shaking for 4 hs

#1 : 1.260 #12 : 1.192

#8 : 0.992 #16 : 0.337

↓ 3.2K for 15' (actually 10' should be enough)

pellets (#16 has smallest pellet)

also has a small pellet and 11 have biggest pellets, are most viscous after suspend the cells

↓ 0.5 ml LETT

↓ completely resuspend cells by pipetting

↓ transfer to eppendorf tubes

↓ boil for ~~90~~ → 120°

↓ 14K 10'

after 10' spinning, #1-5, 7&8, 13-15 did not pellet, boil in for another 3' and repeat spinning.

*1 → *2 → *4

↓ transfer supernatants to new tubes

supernatants *3

after second spin, they form pellets. Freeze them in dr ice/EtoH bath, thaw them, spin for another 10' (5, 7, 14, 15 are treated dry ice/EtoH bath)

↓ 1V ϕ:chl ext.

↓ 2V EtoH

↓ 5' 14K

adjust the supernatants to same volume by LETT

pellets

↓ 70% EtoH Wash

pellets

↓

To Page No. 95

ssed & Understood by me,	Date	Invented by	Date
	3-11-98		9.9.98
		Recorded by	

*4 The lysed cell pellets are different in size: The approximate pellet size (CAPS) are listed in the following table:

ep#	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
PS	400	400	300	250	400	100	250	200	200	200	150	120	100	250	250

The approximate pellet sizes (CAPS) are in microliters.

pellets after 70% EtoH wash are different in size. Their relative size are listed in the following table:

ep#	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
ze	L	L	L	L	L	~~M~~ S	L	M	S	S	S	VS	L	L	L

↓ air dry the pellets O/N.

↓ re-suspend in 200ul TE w/RNase A 9.10

After O/N air dry, the pellets are difficult to be desolved especially the larger pellets. When they are finally desolved (take about 2hs w/ vortexing), they form heavy bfoams while vortexing.

Add another 200ul TE w/RNase A to large pellet tubes namely #1-5, 7, 13-15.

It is amazing that all the pellets seem desolve completely these efforts.

Sma I digestion:

Master Mix for each RXN		16 RXNs
H_2O	6.9 ul	110.4
10X Buffer	1 ul	16
Sma I	0.1 ul	1.6
	8 ul/RXN	128 ul totally.

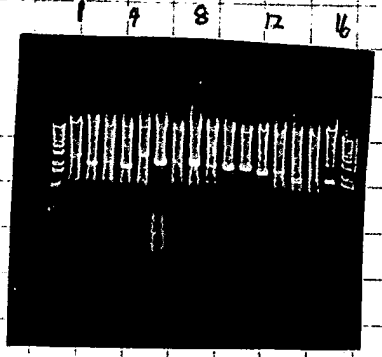
take out 2ul from each sample

↓ add 8ul master mix

↓ R.T for 1h

↓ run on 0.8% agarose TBE

↓ take a picture. (416, ½ sec,



Result analysis:

① Genomic DNA contamination is seri possible solutions ② Decrease the boiling t ⑥ Do not use pepitt to resuspend the cell ⓒ Decrease the Triton-X100 Concentration.

② Most of the preps have enough DNA for future usage. (however 3, 7 and 15 app to have very little DNA (0.025 per band).

possible solutions ④ use higher concentration of chloramphenicol on

To Page No. _____

tnessed & Understood by me,	Date	Invented by	Date
	9-11-98	Recorded by	9-9-10-98